An Unusual New Virus, Possibly of the Potyvirus Group, from Pepper in Nigeria

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ABSTRACT

A virus which induced mottle and foliar rugosity in pepper was mechanically transmitted to several pepper cultivars. Physical properties of the virus were: thermal inactivation point, 62 C; dilution end point, 10^{-3} to 10^{-4}; and longevity in expressed sap about 3 days at 20-25 C. The virus was readily transmitted by *Aphis craccivora, Aphis gossypii,* and an unidentified *Aphis* sp. The virus was not transmitted to any Solanaceous species other than pepper either by mechanical inoculation, grafting, or by the aphid vectors. Electron micrographs of the infectious crude pepper sap indicated that the virus particle was a flexuous-rod, 735 nm × 14 nm. Using both degraded and intact virus, immunodiffusion tests with *antisera of tobacco mosaic virus, pepper mottle virus, potato* virus Y, tobacco etch virus, tobacco rattle virus, and cucumber mosaic virus were unsuccessful. Microprecipitin tests with pepper veinal mottle virus antiserum indicated a possible relationship.

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Virus diseases cause significant production problems for many vegetables in Nigeria, and no crop has been more seriously affected than pepper. A survey in November 1973 showed that the virus-like symptom first observed in certain gardens and farms on pepper plants around Ibadan was widespread in western Nigeria. Personal communication with West African countries also indicated that the pepper virus disease with similar symptoms was an international problem. The symptoms in infected pepper plants did not resemble those reported by Maduewesi (2). A series of transmission experiments were conducted in the greenhouse and in the field using various species and cultivars of herbaceous seedlings to determine the viral nature and properties of the disease agent. This paper reports these investigations.

MATERIALS AND METHODS.—*Mechanical transmission.*—The experiments were performed in the screened glass house sprayed weekly with 0.03 dimethioate insecticide. Inocula were prepared by grinding symptomatic infected pepper leaves in either .01 M phosphate buffer and/or 2% Nicotine (in equal volumes) containing either 38-μm (400-mesh) Carborundum or Celite. In addition to several pepper cultivars, the following plants were inoculated: *Chenopodium amaranticolor* and *C. quinoa; Datura stramonium; Physalis angulata, P. floridana,* and *P. peruviana; Nicotiana tabacum* ('Turkish', 'Samsun NN', '211', 'Johnson Williams', 'BW 3', 'BW 4', and 'White Burley'), *N. glutinosa, N. sylvestris,* and *N. occidentalis; Helianthus annuus; Celosia argentea; Zinnia elegans; Lycopersicum esculentum; Cucumis sativum* and *C. melo; Abelmoschus esculentus; Hibiscus cannabinus* and *H. rosasinensis; Gossypium hirsutum; Vigna unguiculata* ('New Era' and 'Prima'; *V. radiata*), *V. aureus,* and *V. mungo; Voudzeia subterrans; Cajanus cajans; Crotolaria juncea; Glycine max; Petunia hydrida; Solanum nigrum* and *S. melongena.* Noninoculated plants of each species were maintained as controls under similar conditions. Inoculated plants were observed for a period of 40 days and re-isolation of the virus was attempted by mechanical transmission from test plants with or without apparent symptoms to pepper plants.

Vector transmission.—The pattern of spread and epidemic of the disease suggested transmission by insects. Based on the work of Ozolue and Wilson (*unpublished*), potential aphid vectors (*Aphis gossypii, A. craccivora* and an unidentified *Aphis* sp.) were collected in the field and cultured on nonsymptomatic pepper and cotton *(Gossypium hirsutum)* plants in the greenhouse. To ascertain that these aphids were not carrying any latent viruses, nonsymptomatic leaves of these host plants were ground up in appropriate buffer solution and stabilizers, and the crude sap was used for mechanical inoculation on healthy pepper plants. Aphids were starved for 24 hours and then permitted to feed on infected and symptomatic pepper leaves for periods varying from 3 - 30 minutes. Two aphids were transferred to each healthy young pepper plant where they fed from 4 - 240 minutes. Plants on which nonviruliferous aphids have fed for similar periods were used as controls. Both the control and inoculated plants were observed for 21 days.

Properties in crude sap.—Thermal inactivation points, aging in vitro, and dilution end point were determined with crude infected pepper sap and by assaying on healthy pepper plants and common tobacco mosaic virus local lesion assay hosts, like *Chenopodium amaranticolor, C. quinoa* and *Nicotiana glutinosa.*

Electron microscopy.—Negatively stained (2% phosphotungstic acid, pH 7.2) preparations of infected crude sap from diseased pepper plants were observed with the electron microscope. Similarly treated crude sap from healthy pepper was also examined.

Serology.—For serological tests, virus antigen and antisera were tested in precipitin tubes and gel double

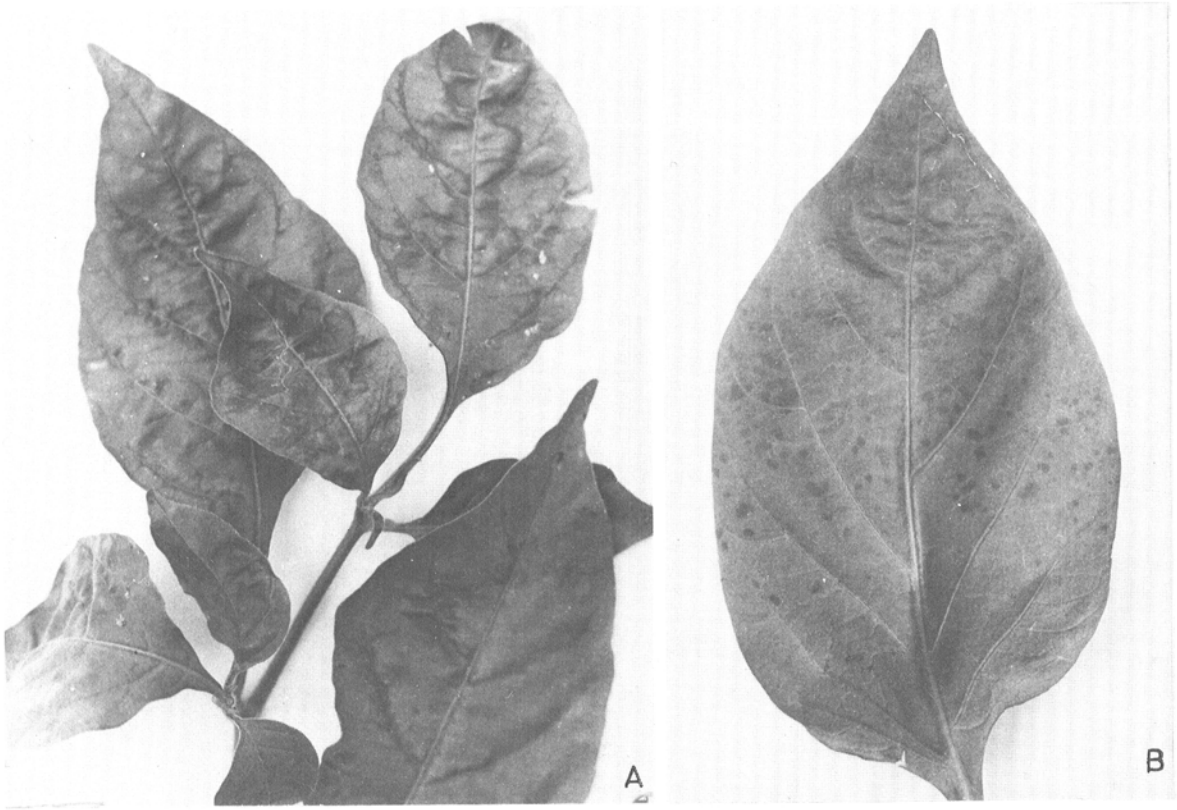

Fig. 1-(A-B). Field symptoms of an unusual virus of the Potyvirus group infecting *Capsicum annuum* in Nigeria, showing **A)** mottling and rugosity of host leaves, and **B)** dark green spots which are characteristics of leaves mechanically inoculated from infected leaves in the field.

diffusion in .075 Ionagar dissolved in distilled water containing: .085% NaCl. Serological tests made in precipitin tubes was done by mixing 1.0 ml of each suitably diluted antigen [purified by method of Brunt and Kenten (1)] and antiserum, and by incubating the mixture at 22 C for 14 hours. With the gel double diffusion test, antigens were either degraded by method of Shepard (4) OR left intact OR not purified at all. The central antiserum depot was surrounded by eight wells. Antisera used included pepper strains of tobacco mosaic virus (TMV), pepper mottle virus (PMV), potato virus Y (PVY), tobacco etch virus (TEV), tobacco rattle virus (TRV), pepper veinal mottle virus (PVMV) and cucumber mosaic virus (CMV) (1, 3, 4, 5, 6, 7). Plates were incubated at about 22 C for about 1 week and examined for veinbanding lines. These tests (micro-precipitin and gel double diffusion) were conducted at Ibadan, Nigeria and at Glasshouse Crops Research Institute in England.

RESULTS.—*Description of the disease.*—Infected *Capsicum frutescens* plants were stunted and leaves developed mild mottling symptoms. In time, the mottling became severe and was accompanied by vein banding. These symptoms were followed by rugosity of the new foliage. Leaf margins started curling upwards (Fig. 1-A).

On *Capsicum annuum,* infected plants developed interveinal yellowing mottling which occasionally developed into vein banding. Leaves of mechanically inoculated plants often developed dark green spots (Fig. 1-B). Fruiting of infected plants were reduced and fruit distortion was evident.

Host range and symptoms.—None of the other thirty-nine herbaceous species and cultivars in the Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Leguminoseae, or Solanaceae became infected. Attempts to transmit the virus mechanically or with aphid vectors to a large number of these species were not successful.

Properties in crude sap.—Sap extracted from infected pepper leaves was infective when heated to 62 C, but not at 65 C. Inoculum diluted at 10^{-3} was infectious but that diluted at 10^{-4} was not. Expressed sap was infectious after 3 days storage at 20-25 C.

Vector transmission.—The virus was transmitted by *Aphis craccivora, Aphis gossypii,* and an unknown *Aphis* sp., collected from melon. Each of the *Aphis* spp. was capable of transmitting the virus acquisition and test feeding of at least 4 minutes each. Vector transmissions occurred only from pepper to pepper and none of the other plant species became infected.

Electron microscopy.—Flexuous rods not found in healthy crude sap were observed in stained preparation of infected crude sap from infected plants. About 60% of the rods were 700 - 750 nm in length, with apparent clusters beginning at about 735 nm × 14 nm (Fig. 2). No inclusion bodies were present in the thin tissue sections which were examined.

Serology.—All of our antisera to the seven viruses were supplied from reliable sources; some from American

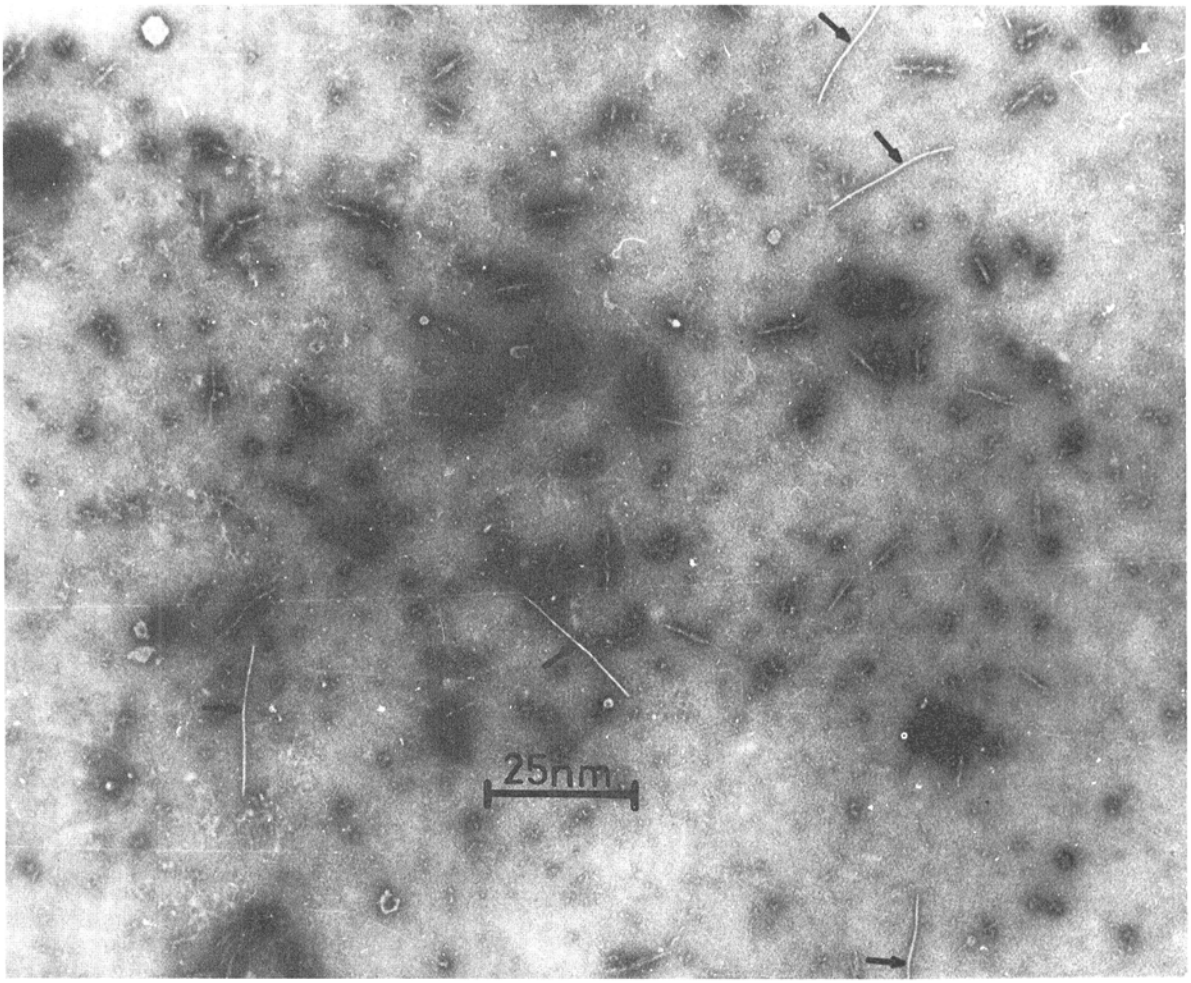

Fig. 2. Electron micrograph of negatively stained crude sap extract from a diseased pepper plant. The flexuous rod structures are particles of the Nigerian pepper virus. These were not present in the sap of healthy plants. × 45,850.

Type Culture Collection, and others from T. A. Zitter, University of Florida, and A. Brunt of Glasshouse Crop Research Institute in England. In microprecipitin tests, clarified sap from infected pepper leaves reacted specifically with a dilution of 1/64 to 1/128 with PVMV antiserum. There was a rapid serological reaction between the purified virus and the PVMV antiserum (1/8,000). However, degraded and intact purified virus failed to react with homologous titers of the following antisera to other viruses [TMV, 1/256 to 1/4,096; PVY, 1/1,024 to 1/4,096; TRV, 1/2,048; TEV, 1/16,000, and CMV, 1/1,024]. In gel double diffusion tests, degraded purified virus reacted with PVMV antiserum, but not with the others.

DISCUSSION.—The successful mechanical transmission of the mottling disease of pepper suggested a viral etiology. Symptoms did not appear in other plant species which were inoculated either mechanically or by grafting with infected pepper tissues or by aphid vectors. No virus was recovered from the symptomless inoculated seedlings, and they are regarded as nonsusceptible.

The relationship of the pepper virus to established virus diseases of pepper (2, 3, 5, 6, 7) except PVMV is not known. The virus did not infect any other member of the Solanaceae tested though most of the known pepper viruses infect at least a few members of this family (7). Even when the infected crude sap of infected pepper plants was diluted to reduce the level of possible inhibitors, symptoms were not induced in any of the test plants other than pepper.

Pepper veinal mottle virus (PVMV) has been reported to be a distinct member of the potato virus Y group (2) as also is a new virus isolated in Arizona (3). Based on serology, symptomatology, and electron-microscopic studies alone, the virus reported here may be a strain of PVMV although cross-reaction serological tests would have to confirm this. Furthermore, the vector relationship between the Ghana, Arizona, and Nigerian isolates is alike since they share the common property of short acquisition and test feeding period and since they are transmitted by the same vector.

The epidemiology of various pepper viruses in Nigeria has not been studied. With this particular virus, all the pepper plants surveyed over a period of 10 months seems to be infected. Whether or not there is an alternate host is not yet known, but there is nowhere in this country that is

free from this pepper virus disease. Aphid vectors serve as a mean of spread, but the original source of infection remains in doubt.

Breeding programs aimed at finding resistant or tolerant lines are already underway at the International Institute for Tropical Agriculture, Ibadan, Nigeria. This is necessary as the yield loss caused by this virus disease appears to be as severe as losses due to similar (not identical) viruses that have been reported in other areas of the world (3).

Based on host range and serological studies, the Nigerian isolate is readily distinguishable from tobacco rattle virus, tobacco etch virus, tobacco mosaic virus, potato mottle virus, potato virus Y and cucumber mosaic virus. On the other hand, based on the same comparison as above, a strain relationship between the Nigerian isolate and PVMV may exist. Further comparison in the same laboratory, and serological cross-reaction tests with antiserum of each virus against highly purified virus preparations would be needed. The quality of flexuousness and the uniformity of length of the virus particles under the electron microscope were such to suggest that this virus is a member of the potyvirus group.

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